Journal of Chromatography A, 1208 (2008) 164–174

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Identification of degradation products in loxoprofen sodium adhesive tapes by liquid chromatography–mass spectrometry and dynamic pressurized liquid extraction–solid-phase extraction coupled to liquid chromatography–nuclear magnetic resonance spectroscopy

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ARTICLE INFO

Article history:
Received 13 May 2008
Received in revised form 20 August 2008
Accepted 22 August 2008
Available online 27 August 2008

Keywords:
LC–MS
Pressurized liquid extraction
Solid-phase extraction
LC–NMR
Identification
Degradation product
Loxoprofen sodium adhesive tape

ABSTRACT

Rapid and unambiguous identification of three degradation products (DP-1, DP-2 and DP-3) found in heat-stressed loxoprofen sodium adhesive tapes (Loxonin tapes) was achieved by LC–MS and dynamic pressurized liquid extraction (PLE)–solid-phase extraction (SPE) coupled to LC–NMR without complicated isolation or purification processes. The molecular formulae of the degradation products were determined by accurate mass measurements and product ion analyses and on-line hydrogen/deuterium (H/D) exchange experiments provided information about changes in the degradation of loxoprofen. To compensate for the low sensitivity of NMR, on-line dynamic PLE–SPE was employed and higher concentrations of degradation products trapped on the SPE column were afforded in a shorter time than they would be in such time-consuming sample preparations as pre-concentration after extraction. The loop-storage procedure was used in the LC–NMR analysis to allow the acquisition of the ^{1}H spectra of the three degradation products in one chromatographic run without affecting the peak separation and to avoid the carry-over of previously eluted DP-1 of high concentration by washing the NMR detection cell prior to the measurement of the DP-2 spectrum. Based on the resulting ^{1}H NMR spectra in combination with the MS results, DP-1 was successfully identified as an oxidation product having an oxodicarboxylic acid structure formed by the cleavage of the cyclopentanone ring of loxoprofen, DP-2 as a cyclopentanone ring-hydroxylated loxoprofen and DP-3 as a loxoprofen L-menthol ester.

1. Introduction

Pharmaceutical products must be safe and effective, and the identification and qualification of degradation products in pharmaceuticals are essential because such degradation products could cause toxic or unexpected pharmacological effects in patients. Stress testing of drug substances and drug products is performed under severe conditions, including elevated temperature, humidity and light irradiation in order to understand their stability characteristics and degradation mechanisms in a short period by analyzing the likely degradation products [1,2]. Stress testing is also important to evaluate the stability in case of excursions outside the label storage conditions [3]. The obtained stability information, including the degradation mechanisms, is also useful in developing the manufacturing processes and in establishing effective packaging configurations and storage conditions, leading to the release of effective and safe pharmaceuticals to the market.

For the identification of degradation products, HPLC hyphenated techniques, especially LC–MS, are now widely utilized. These techniques have allowed rapid identification of the degradation products by providing detailed molecular information without any isolation or purification processes. LC–MS has been the method of choice for the characterization of small quantities of degradation products due to its high sensitivity [4–6] and accurate mass measurement has been very effective in the identification of unknown compounds by providing empirical formulae. On-line hydrogen/deuterium (H/D) exchange LC–MS using deuterium oxide as a component of the mobile phase has played a key role in the dif-

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doi:10.1016/j.chroma.2008.08.076

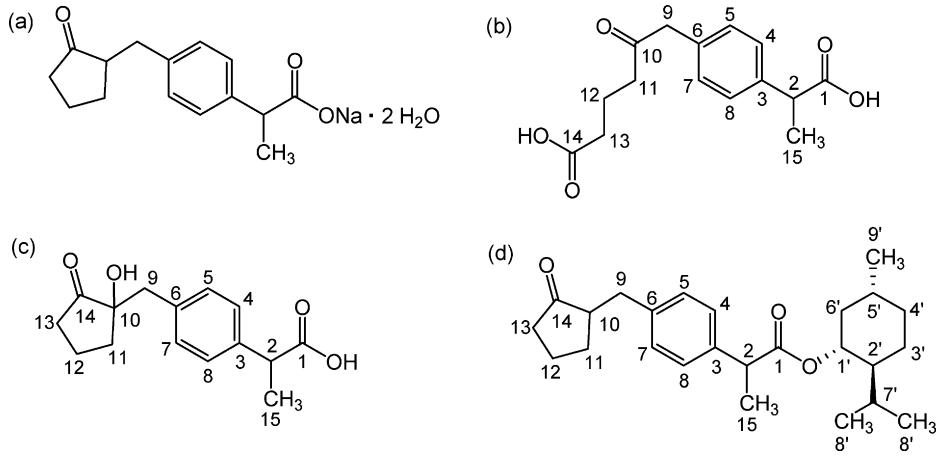

Fig. 1. Chemical structure of (a) loxoprofen sodium hydrate and degradation products in Loxonin tapes: (b) DP-1; (c) DP-2 and (d) DP-3. The atom numbering used for the description of NMR results.

ferentiation of isomeric structures by identifying labile hydrogen atoms, especially in such cases as the identification of structural isomers which are difficult to distinguish by their accurate mass or product ion spectra [7,8]. However, these MS data alone cannot always provide unequivocal identification of the compounds and therefore other spectroscopic and spectrometric analyses are required for a definite structure elucidation. Recently, LC–NMR has been increasingly applied to the identification of degradation products [9–16]. LC–NMR experiments can be performed in on-flow, stopped-flow and loop-storage modes. However, stopped-flow or loop-storage LC–NMR analysis would be required for a structural analysis due to the low sensitivity of NMR. The loop-storage method is useful when there are several peaks of interest in the chromatogram because the chromatographic separation is not interrupted by the stopping and starting of the mobile phase flow. Moreover, the contamination of samples with a previously eluted peak, which is often observed in the stopped-flow measurement of closely eluting peaks, can be avoided using the loop-storage method by reducing the diffusion effects with much smaller inner diameter storage loops compared to the large inner diameter cavity of the NMR detection cell. Additional washing of the NMR cell can also be applied before the measurement of the stored peaks to avoid carry-overs of the samples in the NMR cell, if necessary, in cases involving the measurement of peaks with large concentration differences [17].

Loxoprofen sodium adhesive tape (Loxonin tape) is a percutaneous absorption-type analgesic and anti-inflammatory drug marketed only in Japan containing loxoprofen sodium hydrate, monosodium 2-{4-[(2-oxocyclopentyl)methyl]phenyl}propanoate dihydrate, with the chemical structure as shown in Fig. 1a. Loxoprofen sodium is a prodrug in the class of short-acting nonsteroidal anti-inflammatory drugs (NSAIDs) and is widely used for the treatment of pain and inflammation as an orally administered agent [18,19]. Loxoprofen is converted to a *trans*-OH form (2-{4-[(*trans*-2-hydroxycyclopentyl)methyl]phenyl}propanoate), which is an active metabolite, by a ketone-reducing enzyme existing mainly in the liver and kidney but also in the dermal layer of the skin [20]. The anti-inflammatory and analgesic activities of the percutaneous preparation of loxoprofen sodium have been reported to be comparable to those of the oral administration [21,22].

In the analysis of percutaneous preparations, sample extraction using conventional techniques such as shaking extraction are time and solvent consuming, and the concentration of the resulting extract would be needed for the preparation of a sample to be subjected to insensitive analyses such as LC–NMR measurements in the identification of low-level degradation products present in the formulation. Moreover, special care is required during the concentrating processes in cases where the degradation products are labile, since further degradation of the degradants can occur. As one of extraction techniques, pressurized liquid extraction (PLE) has been used to obtain a more concentrated extract in less time than conventional techniques. This technique can be coupled to other analytical steps, such as pre-concentration, filtration, chromatographic separation and detection [23–25], and on-line coupling of PLE and solid-phase extraction (SPE) pre-concentration enables subsequent HPLC analysis with a low-sensitivity detector by providing a more concentrated sample.

In the present work, the chemical structures of three degradation products in heat-stressed Loxonin tapes were successfully elucidated by LC–MS and PLE–SPE–LC–NMR. The on-line H/D exchange method was applied in the LC–MS analysis to determine the number of H/D exchangeable functional groups on the degradant molecules. Dynamic PLE and SPE were coupled on-line to LC–NMR and the loop-storage method was employed to acquire the ^{1}H NMR spectra of the three degradation products in one chromatographic run. As a result, the ^{1}H NMR spectra of the degradation products were successfully obtained and an accurate identification was completed.

2. Experimental

2.1. Chemicals and reagents

Loxonin tapes (7 × 10 cm, with loxoprofen sodium content of 50 mg as an anhydrate) were provided by Lead Chemical (Toyama, Japan). These tapes were hermetically packed with an aluminum composite film using the seven sheets supplied in each package and these packages were stored at 80 °C up to 3 weeks. Loxoprofen sodium hydrate was synthesized by Daiichi-Sankyo (Tokyo, Japan). Acetone and methanol of special grade, trifluoroacetic acid (TFA) for amino acid sequence analysis, acetonitrile of HPLC grade and Celite No. 500 (1.5 μm particle size) were purchased from Wako (Osaka, Japan), tetrahydrofuran (THF) of HPLC grade was purchased from Kanto (Tokyo, Japan), and deuterium oxide (D_2O, 99.9% D) and deuterated acetonitrile (CD_3CN, 99.8% D) were purchased from Cambridge Isotope Labs. (Andover, MA, USA). Water was purified with a Milli-Q Gradient A10 system (Millipore, Bedford, MA, USA).